Notch signalling: **You make me feel so glial**

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The signals that instruct neural stem cells to differentiate into glia have long proved elusive. Surprising new evidence suggests that this role could be fulfilled by Notch signalling, previously thought to be a general inhibitor of stem cell differentiation.

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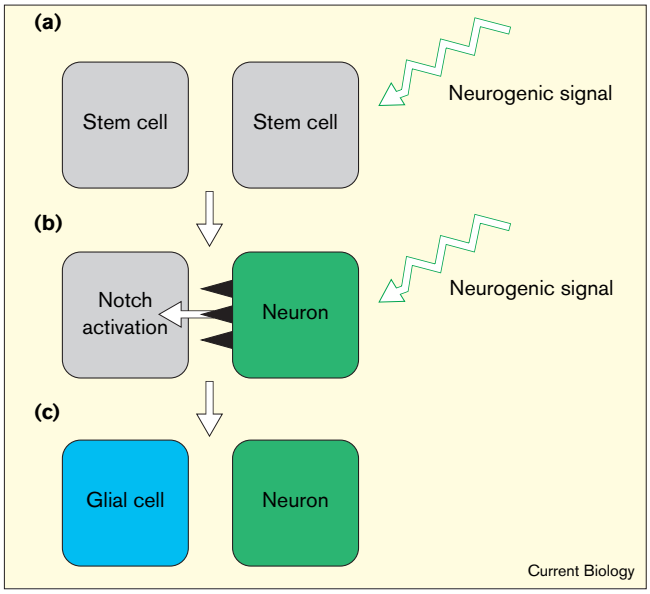
Neurons could not function without their close neighbours, the glial cells which ensheath and insulate their axons and provide physical support. These two cell types are intimately associated right from the start, emerging from the same population of multipotent stem cells during development. In most cases the neurons emerge first, in response to instructive signals in the local enviroment. This poses a problem: why do some of the stem cells ignore these neurogenic signals and later differentiate into glial cells? The answer lies in feedback signals; neighbouring stem cells communicate with each other to ensure that they do not follow the same fate. One such feedback signal comes from the transmembrane protein Delta, which activates signalling through the Notch receptor in neighbouring cells. Notch signalling was first described in *Drosophila*; it is now known to be conserved in vertebrates, where it regulates the patterning of cell fates during the development of several different tissues.

During vertebrate neurogenesis, the Notch receptor is expressed ubiquitously whilst its ligand Delta appears transiently on the surface of newly differentiating neurons [1]. Neighbouring stem cells, which are exposed to this Delta signal, tend to resist the prevailing neurogenic signals, ultimately becoming glia (see Figure 1). Resistance to the neurogenic signals is dependent on the activation of Notch by Delta: targeted deletion of Notch leads to massive overproduction of neurons and early death of the embryo. Analysis of these Notch null mice is complicated by the fact that Notch signalling regulates many cell fate decisions, not just the choice between neurons and glia. More informative approaches have been based on localised activation or interference with the Notch pathway. In chick and *Xenopus*, for example, retroviral vectors have been used to deliver active or dominant-negative forms of Delta to accessible regions of the embryo, such as the retina [2,3].

Until recently, the accumulating data supported a general model in which the Notch signal protects stem cells from *all* differentiation signals, rather than merely inhibiting neural differentiation. According to this view, the stem cell will ultimately choose its fate according to whichever instructive factors prevail when it is released from the influence of Delta. Hence, blocking the Notch pathway at different times during the development of the retina results in the overproduction of different neural cell types. In each case, this is accompanied by a deficiency of glial cells, which are normally the last cell type to be born [2].

This raises several questions. Is glial differentiation a default pathway that is chosen in the absence of any other instruction? Alternatively, could there be an instructive factor, as yet undiscovered, that directs glial differentiation? If so, does Notch behave according to the model outlined above, protecting stem cells from the glial differentiation signal? Recent studies have shed new light on these issues. *In vivo* analysis of the forebrain has provided an example in which Notch clearly does *not* inhibit glial differentiation [4]. Indeed, it now seems that Notch can act in quite the opposite way in the case of the

Figure 1



(a) Neural stem cells (grey) are exposed to neurogenic signals in the local environment. **(b)** As the first of the stem cells starts to differentiate into a neuron (green), it expresses Delta (black) on the cell surface. Delta activates Notch signalling in neighbouring cells. **(c)** Cells exposed to Delta ignore the neurogenic signal and later differentiate into glia (blue).

stem cells of the peripheral nervous system. A rigorous *in vitro* analysis, carried out by Morrison *et al.* [5], has shown that Notch itself can trigger neural crest stem cells to differentiate into glia.

The forebrain has become accessible to genetic manipulations thanks to a new technique that uses ultrasound to guide the delivery of retroviral vectors. The forebrain contains a specialised glial subtype called the radial glia. These cells serve as a scaffold along which the newly emerging neurons will migrate, and for this reason they are born before rather than after the neurons. Gaiano and colleagues [4] found to their surprise that they could increase the number of these very early born glial cells by delivering an activated form of Notch to the stem cells. This is in contrast to the systems that had been studied previously, where Notch inhibits differentiation. This work makes it clear that Notch does not oppose glial differentiation, but still leaves open the question of what the putative glial instructive factor is.

Neural crest cells migrate from the neural tube and aggregate to form the sensory and autonomic ganglia of the peripheral nervous system. These postmigratory populations include stem cells that can differentiate into neurons in response to bone morphogenetic proteins (BMPs) — intercellular signalling proteins of the transforming growth factor β family — secreted by neighbouring tissues. Some stem cells do not respond to this strong neurogenic signal, instead differentiating later into glia [6]. Just as in the retina, retroviral manipulations *in vivo* have revealed that this subset of neural crest stem cells depends on Notch signalling to protect them from a neural fate [5].

Morrison *et al.* [5] were able to take advantage of a recent breakthrough in their lab: the ability to prospectively isolate neural crest stem cells and study them *in vitro* [7]. This means that, unlike the situation *in vivo*, it is possible to control the presence or absence of instructive differentiation factors. Morrison *et al.* [5] reasoned that, if Delta is protecting stem cells from differentiation, then they should become susceptible to whichever instructive signal prevails after Delta is withdrawn. They tested this by exposing stem cells transiently (for 24 hours) to a soluble Delta–Fc fusion protein, which is able to activate Notch signalling when clustered with an Fc antibody. The cells were then challenged with the strong neurogenic instructive factor BMP2. Surprisingly, nearly all the cells ignored the neurogenic instruction and instead differentiated rapidly into glia.

Because the cells were in culture, Morrison *et al.* [5] were able to perform a rigorous clonal analysis, counting the number of each differentiated cell type that emerges from an individual stem cell. These studies demonstrated that the increase in glia was not a consequence of selective proliferation of glial cells or death of neurons, but rather represented a bias in the fate decisions of the stem cells. A final piece of evidence came from measuring the kinetics of differentiation: even transient exposure to Delta commits cells to a glial fate more rapidly than any factor that has yet been found.

This work confirms that Notch signalling inhibits neurogenesis in the peripheral nervous system, just as it does in all the other neurogenic regions that have been studied. Rather than maintaining stem cells in an uncommitted state, however, the Delta signal commits neural crest stem cells to a glial fate. This could have important clinical implications. The transplantation of neural stem cells to repair damaged nervous systems now seems a realistic goal [8]. Success may rely on manipulating cell signalling in the starting population of donor stem cells. For example, it has been suggested that Notch activation might be useful for maintaining a stock of multipotent stem cells. These could later be differentiated into the neural subtype of choice by switching off Notch and switching on the appropriate differentiation signal. According to the new studies, however, activating Notch might not always maintain pluripotentiality, but instead might generate populations of differentiated glia. This could yet prove to be a useful tool, albeit in a different way from that originally envisaged. A major problem in neuronal replacement therapies is the difficulty of integrating transplanted neurons into host tissue. Accompanying the transplant with glial cells may support their survival and integration.

Notch not only influences the choice between neural and glial fates; it also regulates a wide range of different binary cell fate decisions throughout development. The original idea, that Notch mediates a general ‘do not differentiate’ signal, rather then specifying any particular cell lineage, made it easy to understand how it could be used for regulating all these different cell types. Attractive as this idea is, it now seems that it does apply universally. The results described above, and other recent studies from a different tissue, the human epidermis [9], indicate that Notch can act to promote stem cell differentiation. How can cells respond in such different ways to the same signal? One clue has come from a recent study of the target genes of the Notch pathway [10]. Notch signalling activates transcription of genes for several different members of the Hes family of transcription factors. Forced expression of *Hes-1* can inhibit differentiation of both neural and glial cells from retinal stem cells, whilst *Hes-5* has recently been shown to drive glial differentiation [10].

Although Notch signalling has evidently been conserved throughout much of evolution, it has clearly been adapted for use in different ways by different cell types. Are there any universal principles that underlie the way that neural stem cells respond to Notch signals? The only safe prediction is that future studies will uncover more surprises.

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